

Research article

Role of soy protein concentrate on oxidative stress and DNA fragmentation in streptozotocin-induced diabetic rats

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Abstract

Diabetes mellitus is currently a major public health concern with many pathophysiological alterations; this study aimed to evaluate the antidiabetic and protective potential of soy protein concentrate (SPC) against diabetes mellitus induced by streptozotocin (STZ) in adult male Spargue Dawely rats. The hyperglycemic groups were made with a single (ip) dose of STZ (55mg/kg b.w.). Five groups of rats were used; normal control group, SPC (200 mg/kg/day) treated group, hyperglycemic untreated group, hyperglycemic-diamicrone® 30MR (10 mg/kg/day) treated group and hyperglycemic-SPC (200 mg/kg/day) treated group. The experiment lasted for six weeks. The results revealed significant increases in blood glucose, hepatic and renal MDA and NO contents concomitant with significant depletion of GSH content and decreases in insulin level, SOD and CAT activities in the diabetic group; moreover, significant increases in serum ASAT, ALAT, GGT, urea, creatinine, total cholesterol, LDL-cholesterol and triglycerides matched with a significant reduction in HDL-cholesterol and body weight values were noticed compared to control values. Interestingly, significant improvements in all biochemical, DNA fragmentation and histological determinations were observed in the diabetic-SPC treated group compared to diabetic group, especially DNA fragmentation percent. Also, diamicrone® significantly ameliorated the severity of STZ induced changes in the above mentioned parameters with the exception of DNA fragmentation which was severely elevated than diabetic group; reflecting the necessity to find more safer alternative treatments. In conclusion: the relative hypoglycemic and highly protective effects of SPC against STZ-induced diabetes mellitus may be attributed to its antioxidant and free radical scavenging mechanisms that due to its higher contents of thiol-rich amino acids and antioxidant ones.

Introduction

Diabetes mellitus (DM) was reported to affects about 150 million people worldwide, and this is expected to be doubled in the next 20 years [1]. Diabetes mellitus is a syndrome of disordered metabolism with abnormally elevated blood glucose levels (hyperglycemia) [2]. There are two forms of diabetes, type 1 diabetes (diminished production of insulin) and type 2 diabetes (impaired response to insulin and β -cell dysfunction). Both types of diabetes lead to hyperglycemia, increase in urine production, increased fluid intake, blurred vision, increased weight loss, lethargy, and changes in energy metabolism [2]. DM exhibits excessive oxidative stress and highly reactive oxygen species (ROS) production in pancreatic islets due to persistent and chronic hyperglycemia, thereby decreases the activity of the antioxidative defense system, and thus promotes free radical generation [3]. Oxygen free radicals have been

suggested to be a contributory factor in complications of DM [4]. It is demonstrated that DM is an oxidative stress-related disorder and the antioxidants may be useful in preventing it [5]. Therefore, the supplementation with antioxidants may have a chemoprotective role in diabetes [6]. Many plant extracts and their products have been shown to have significant antioxidant effect in treating many kinds of diseases [7]. The use of medicinal plants for the treatment of human diseases has increased considerably worldwide [8].

STZ induces diabetes by generating reactive oxygen species (ROS), which leads to islet cell destruction in experimental animals [9]. It has various biological actions, including the production of acute and chronic cellular injury, carcinogenesis, teratogenesis and mutagenesis [10]. Several reports showed that the use of plants is also helpful in prophylaxis or treatment of diabetes. Given that, herbal medicine possesses significant efficacy, low incidence of side effects, low

cost and relative safety [11], while synthetic antidiabetic agents can produce serious side effects, as hypoglycemic coma and disturbances of the liver and kidneys [12].

Soy rich food is one such example which is catching the attention of a common man as well as researchers due to its numerous health benefits. Soy beans are a rich source of isoflavones. Soy rich food and isoflavones are of great interest when it comes to medicinal foods. The various health benefits which are claimed by soybean consumption in any form are cholesterol lowering, anticancer, antioxidant and chelating effects. Among all these beneficial effect, the most extensively researched upon properties are its cholesterol lowering effect, its role in prevention of osteoporosis and its role as an anticancer agent [13]. The current study was conducted to evaluate the antidiabetic potential of soy protein concentrate (SPC) against diabetes mellitus induced by streptozotocin (STZ) in adult male Spargue Dawely rats.

Experimental

Materials and methods

This study was conducted on adult male Spargue Dawely rats, weighing 120-150g, obtained from Animal House Colony, National Research Centre, Dokki, Egypt. The animals were housed in suitable plastic cages for one week for acclimation. All animals received human care in compliance with the standard institution's criteria for the care and use of experimental animals according to the NRC ethical committee (committee number FWA 00014747).

Chemicals

Streptozotocin (STZ, Sigma 85882), Sodium citrate (Sigma C0909) and Citric acid (Sigma C1909) were purchased by Egyptian International Center for Import, 22 Abu Zer El Ghafary St., Nasr City Cairo, Egypt.

Diamicrone® 30 MR (manufactured by Servier Egypt, 67 El Horreya St., Helliopolis, Cairo, Egypt) was obtained from a local pharmacy, Cairo, Egypt.

Soy protein concentrate (60%) was purchased from Nantong Sun-Green Bio-Tech Co., China. According to manufacturers claim this soy protein concentrate contains (as percentage) aspartic 5.52, threonine 2.07, serine 2.44, glutamic 9.07, proline 2.23, glycine 2.25, alanine 2.53, valine 2.84, methionine 0.75, isoleucine 2.37, leucine 3.85, tyrosine 2.24, phenylalanine 2.83, histidine 1.37, lysine 3.33, arginine 3.72 and cystine 0.69.

Induction of type II diabetes mellitus

After fasting 16 hours, animals were intraperitoneally injected with streptozotocin (55mg/kg b.w.) dissolved in ice cold 0.1 M sodium citrate buffer (20ml of 0.1 M sodium citrate with 30ml of 0.1M citric acid, pH=4.0) followed by oral administration of 2-3 ml sucrose

solution 10% (w/v) for one day next. Animals were fasted overnight and one drop blood sample was obtained by nicking the lateral tail vein using a sterile surgical scissors and immediately the blood glucose level was determined using Gluco Dr SUPER SENSOR AGM-2200, Korean glucometer, and its test strips. Animals with blood glucose level above 240 mg/dl were considered to be diabetic [14].

Study animal groups

Both normal and diabetic rats were rearranged randomly in 5 groups (10 animals each); 1) normal animals administrated orally with distilled water acting as negative control; 2) normal animals subjected to oral administration with 200 mg/kg/day of soy protein concentrate (SPC) for six weeks ; 3) STZ-diabetic animals without any treatments acting as positive control; 4) STZ-diabetic animals administrated with 10 mg/kg/day Diamicrone® 30 MR drug, dissolved in distilled water, for six weeks; and 5) STZ-diabetic animals subjected to administration with 200 mg/kg/day of SPC for a similar period.

Blood sampling

At the end of the study period, animals were weighed then fasted overnight and blood glucose level of each animal was determined again with the GlucoDr set using a tail blood drop; and following diethyl ether anesthesia blood samples were immediately withdrawn from the retro-orbital plexus using heparinized and sterile glass capillaries; blood samples were cool centrifuged at 3000 rpm for 10 minutes using (IEC centra-4R, International Equipment Co., USA) and the sera were separated immediately, divided into aliquots and stored at -70°C for further biochemical measurements as soon as possible.

Tissue sampling

After blood collection, the animals were rapidly sacrificed; then both the liver and kidneys of each animal were dissected out. The left kidney and portion of the liver were washed with saline, dried, rolled in a piece of aluminum foil and stored at -80°C for homogenization and DNA fragmentation determinations. A specific weight (g) from each liver and kidney was subjected to homogenization in ice-cold phosphate buffer (50 mM, pH 7.4) to give 10% homogenate (w/v) and dilution factor equal 10; then the homogenate was centrifuged at 5000 rpm for 20 minutes to remove the nuclear and mitochondrial fractions. The supernatant was divided into aliquots and stored at -80°C till the determination of the biochemical measurements.

Biochemical assessments in serum

Insulin level was determined in serum by enzyme-linked immuno-sorbent assay (ELISA) using kits manufactured

by Immunospec Corporation, Suite103 Canoga Park, Ca91303, USA. All other biochemical measurements were carried out using Shimadzu spectrophotometer (UV – Vis. 1201), Japan. Serum total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides levels were determined photometrically using the instruction manual of DiaSys reagent kits purchased from DiaSys Diagnostic System GmbH, Germany. ASAT, ALAT and GGT activities were determined using kits purchased from Human Gesell Schaft fur Biochemical und Diagnostic mbH, Germany. Serum urea and creatinine levels were determined using kits obtained from Biodiagnostic, Dokki, Giza, Egypt.

Biochemical assessments in homogenates

Reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and nitric oxide (NO) values in liver and kidney homogenates were determined spectrophotometrically using reagent kits obtained from Biodiagnostic, Dokki, Egypt. Malondialdehyde (MDA) is the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems and used as an indirect index for lipid peroxidation. MDA in liver and kidney homogenates was determined chemically according to the method described by Ruiz-Larrea *et al.* [15]. 0.5 ml liver homogenate supernatant was added to 4.5 ml working reagent (0.8 g thiobarbituric acid was dissolved in 100 ml perchloric acid (10%) and mixed with trichloroacetic acid (20%) in volume ratio 1 to 3, respectively). In a boiling and shaking water bath, the sample-reagent mixture was left for 20 minutes, then cooled at room temperature and centrifuged for 5 minutes at 3000 rpm. The absorbance of the clear pink supernatant was measured at 535 nm against reagent blank (0.5 ml distilled water + 4.5 ml TBA working reagent). MDA nmol/g tissue according to the formula $MDA \text{ (nmol/g tissue)} = \left[\frac{A_{535} \times 10^9}{(1.56 \times 10^5) \times 10^3} \times AD \right] \times 10$; where, $1.56 \times 10^5 \text{ M}^{-1}\text{L}^{-1}\text{cm}^{-1}$ (extinction coefficient of MDA), AD (assay dilution).

DNA fragmentation percentage

The degree of DNA fragmentation was determined by separating the cleaved DNA from the intact chromatin by centrifugation and measuring the amount of DNA present in the supernatant and pellet using the diphenylamine assay according to the quantitative method used for grading the DNA damage [16]. The degree of DNA fragmentation refers to the ratio of DNA in the supernatant to the total DNA in the supernatant and pellet. The liver tissues were lysed in 0.5 ml of hypotonic lysis buffer containing 10 mM Tris-HCl (pH 8), 1 mM EDTA and 0.2% Triton X-100, and centrifuged at 14,000 ×g for 20min at 4 °C. The pellets were resuspended in hypotonic lysis buffer. To the resuspended pellets and the supernatants, 0.5 ml of 10% trichloroacetic acid (TCA) was added. The samples were centrifuged for 20 min at

10,000 ×g at 4 °C, and the pellets were suspended in 500 µl of 5% TCA. Subsequently, each sample was treated with a double volume of diphenylamine (DPA) solution [200mg DPA in 10 ml glacial acetic acid, 150 µl of sulfuric acid and 60µl acetaldehyde] and incubated at 4°C for 48h. The proportion of fragmented DNA was calculated from the absorbance reading at 578 nm using the following equation:

$$\text{DNA fragmentation (\%)} = \left[\frac{A_{\text{supernatant}}}{A_{\text{supernatant}} + A_{\text{pellet}}} \right] \times 100$$

Histological examination

The right kidney and another portion of the liver were placed in 10% (v/v) formalin-saline buffer solution followed by sectioning and staining with hematoxyline and eosin for light microscopic examination.

Statistical analysis

The obtained data are presented as mean ± standard error after they were subjected to one way analysis of variance (ANOVA) followed by (Tukey) post hoc test at levels of $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ according to Steel and Torrie [17] using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

Results and Discussion

Results

Data in table (1) shows a significant decrease in insulin level coupled with a significant increase in blood glucose in diabetic group when compared with the control group. Interestingly, administration of diabetic rats with SPC succeeded to improve both insulin and glucose serum levels towards normal values as it significantly increased insulin and significantly decreased the glucose levels compared to diabetic animals. This reflects the analogue hypoglycemic potential of SPC similar to that of the pharmaceutical drug Diamicon®.

Table 1. Blood glucose and serum insulin of normal, streptozotocin-induced diabetic and diabetic- SPC treated rats as compared to each other.

	glucose (mg/dl)	Insulin (ng/ml)
Control	113±9 ^{cd}	2.10±0.10 ^b
SPC	118±4 ^d	2.11±0.07 ^b
Diab	391±15 ^a	0.40±0.0z ^c
Diab+Diamicon®	140±10 ^c	2.45±0.1 ^b
Diab+ SPC	192±2.3 ^b	2.35±0.34 ^b

Data are presented as mean ±S.E; within each column, means with different superscript letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Tukey test.

Data in table (2) show the effects of different treatments on hepatic and renal MDA and nitric oxide (NO) of the animals. On comparison with the control group, STZ – induced diabetes significantly increased the levels of hepatic and renal MDA and NO. SPC intake alone had no significant effect on these parameters. The diabetic animals that given SPC or Diamicron® showed significant decreases in hepatic and renal MDA and NO levels as compared to the diabetic group.

Table (3) shows the effect of different treatments on some different antioxidants such as GSH, SOD and CAT in hepatic and renal tissues. STZ treatment resulted in significant decrease in GSH, SOD and CAT values when compared to values of the control group, while the administration with SPC alone produced a non-significant effect in these parameters. The treatment of diabetic rats with SPC or Diamicron® ameliorated the decrement in GSH, SOD and CAT values that induced by STZ treatment.

With regard to results of DNA fragmentation percentage, the diabetic group showed a significant ($p \leq 0.01$) increase in the percent of DNA fragmentation as compared to control rats. Treatment of diabetic rats with soy protein resulted in a remarkable significant ($P \leq 0.001$) decrease in the percent of DNA fragmentation reaching a level close or non significant to that of control (Figure1).

Unfortunately, treatment of the diabetic group with Diamicron® 30MR didn't improve the percent of DNA fragmentation but led to a significant elevation ($P < 0.01$)

in hepatic DNA fragmentation, compared to that of diabetic group.

The effects of STZ and SPC on animal's body weight gain (BWG) and some parameters of lipid profile are depicted in table (4).

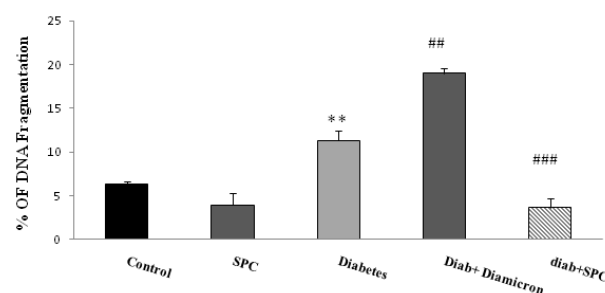


Figure 1. Shows the effect of soy protein concentrate in comparison to Diamicron® on hepatic DNA fragmentation of control and diabetic male albino rats. (**) is significantly different from control group ($p \leq 0.01$) and (#) is significantly different from diabetic group (# at $p \leq 0.05$, and ## at $p \leq 0.01$).

The data show that STZ injection induced significant increases in total cholesterol, LDL-cholesterol and triglycerides levels, and significant decrease in HDL-cholesterol level and body weight gain (BWG) compared with the control group. The mentioned parameters showed insignificant changes in rats treated with SPC alone. The treatment with SPC or Diamicron® could protect these parameters against the above mentioned effects of STZ.

Table 2. MDA and nitric oxide (NO) levels in hepatic and renal homogenates of normal, streptozotocin-induced diabetic and diabetic- SPC treated rats as compared to each other.

	MDA (nmol/g tissue)		NO (μ mol /g tissue)	
	Liver	Kidney	Liver	Kidney
Control	77.1±4.3 ^b	42±2.1 ^d	53±4.2 ^c	2.9±0.10 ^b
SPC	73.4±5.2 ^b	39±1.26 ^d	55±4.2 ^c	2.9±0.12 ^b
Diab	149±11.3 ^a	206±4.2 ^a	71±2.7 ^a	4.3±0.32 ^a
Diab+Diamicron®	103±4.2 ^d	175±3.4 ^b	62±6.9 ^b	4.1±0.31 ^a
Diab+ SPC	87.7±2.7 ^c	84.4±1.7 ^c	58±3.1 ^c	3.4±0.13 ^b

Data are presented as mean ±S.E; within each column, means with different superscript letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Tukey test.

Table 3. GSH, SOD and CAT in both hepatic and renal homogenates of normal, streptozotocin-induced diabetic and diabetic- SPC treated rats as compared to each other.

	GSH (mg/g Tissue)		SOD (U/g Tissue)		CAT (nmol/g Tissue)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Control	79±3.1 ^a	55±0.6 ^a	3178±28 ^a	3823±65 ^a	2.53±0.11 ^b	1.73±0.05 ^a
SPC	77±2.9 ^a	59±1.8 ^a	3227±84 ^a	3613±42 ^a	2.78±0.09 ^a	1.66±0.04 ^a
Diab	45±1.3 ^c	35±1.3 ^c	2306±40 ^c	2498±28 ^d	1.42±0.12 ^d	1.24±0.03 ^b
Diab+Diamicron®	58±2.1 ^b	41±1.4 ^b	2470±56 ^c	2767±31 ^c	1.91±0.14 ^c	1.31±0.05 ^b
Diab+ SPC	71±4.5 ^a	46±1.5 ^b	2961±48 ^b	3197±35 ^b	2.03±0.13 ^c	1.62±0.02 ^a

Data are presented as mean ±SE; within each column, means with different superscript letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Tukey test.

Table 4. Serum lipid profile and body weight gain (BWG) of normal, streptozotocin-induced diabetic and diabetic- SPC treated rats as compared to each other.

	Chol (mg/dl)	Trigl (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	BWG (g/100g b.w)
Control	96±3.9 ^d	67±2.3 ^c	32.4±5.1 ^a	59.7±3.2 ^b	34±2.1 ^a
SPC	91±3.6 ^d	61±7.9 ^c	33±1.44 ^a	58.3±2.2 ^b	35±1.9 ^a
Diab	210±5.2 ^a	121.4±6 ^a	27.8±2.3 ^b	71.6±3.8 ^a	7.6±3.6 ^c
Diab+Diamicron®	172±11 ^b	82±3.5 ^b	31.4±5.1 ^a	63.4±4.1 ^b	24±5.8 ^b
Diab+ SPC	148±10 ^c	86±4.8 ^b	30.2±4.9 ^a	62.4±5.9 ^b	25±2.3 ^b

Data are presented as mean ±S.E; within each column, means with different superscript letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Tukey test.

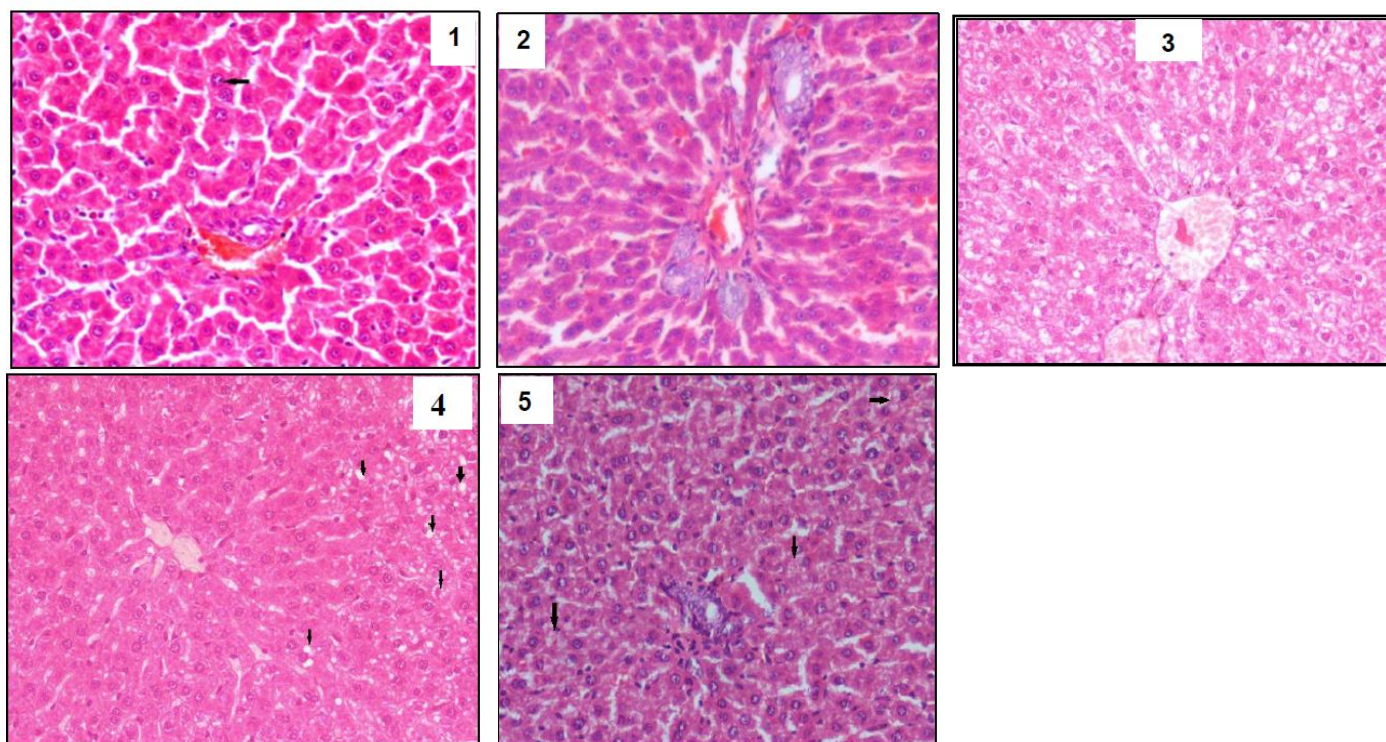


Figure 2. Liver sections (H&E, x400) show the effect of SPC and Diamicron®30 MR administration on the hepatic tissue of STZ-induced diabetic rats in compare to that of normal control. 1) normal control animals group shows normal appearance of the hepatocytes (arrows); 2) normal-SPC treated animal group shows normal appearance of the hepatocytes; 3) diabetic animal group shows wide cloudy and hydropic degeneration in most of the hepatocytes; 4) diabetes-diamicron® 30 MR treated animal group shows cloudy hydropic degeneration and few steatosis vacuoles (arrows); and 5) diabetes-SPC treated animal group showed mild cloudy degeneration in some of the hepatocytes (arrows) in otherwise normal hepatic tissue.

The results in table (5) indicated that STZ injection induced significant elevations in ASAT, ALAT, GGT, urea and creatinine values as compared with the normal values of the control group. The treatment with SPC alone resulted in no significant effects on these parameters as compared with the normal rats. Administration of SPC or diamicron® 30MR to diabetic rats ameliorated significantly the diabetes- induced changes in the mentioned parameters.

The light microscopical examination of the liver sections from the control rats revealed normal hepatocytes architecture (Figure 2). The liver sections obtained from

normal-SPC treated animal group showed normal appearance of the hepatocytes. In contrast, the liver sections obtained from diabetic animal group showed wide cloudy and hydropic degeneration in most of the hepatocytes. Diabetes-diamicron® 30 MR treated animal group showed cloudy hydropic degeneration and few steatosis vacuoles but diabetes-SPC treated group showed mild cloudy degeneration in some of the hepatocytes in otherwise normal hepatic tissue.

The light microscopical examination of the kidney sections from both control and normal-SPC treated groups showed normal appearance of the glomeruli and

the tubules (Figure 3). Diabetic untreated animal showed moderately shrunken glomeruli, dilated tubules, wide tubular cells and some intratubular casts. In Diabetic-Diamicron®30 MR treated group, the glomeruli showed mild thickening of the basement membrane, occasional

tubules are dilated and some of the tubular cells showed vacuolization, while diabetic-SPC treated group showed normal appearance of the glomeruli, few of the tubules showed mild dilatation, some of the tubular cells showed vacuolization of their cytoplasm.

Table 5. Hepatic and renal functions of normal, streptozotocin-induced diabetic and diabetic- SPC treated rats as compared to each other.

	ALAT (U/L)	ASAT (U/L)	GGT (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control	118±6.2 ^d	290±9.6 ^b	1.63±0.04 ^c	36±3.5 ^c	0.67±0.04 ^d
SPC	123±3.6 ^d	279±7.3 ^b	1.71±0.03 ^b	37±1.1 ^c	0.71±0.02 ^d
Diab	261±5.5 ^a	376±9.4 ^a	1.96±0.04 ^a	87±2.5 ^a	2.33±0.09 ^a
Diab+Diamicron®	233±8.4 ^b	293±8.3 ^c	1.85±0.05 ^a	63±1.3 ^b	1.52±0.11 ^b
Diab+ SPC	189±2.8 ^c	259±7.6 ^b	1.77±0.02 ^b	44±2.6 ^c	1.16±0.13 ^c

Data are presented as mean ±S.E; within each column, means with different superscript letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Tukey test.

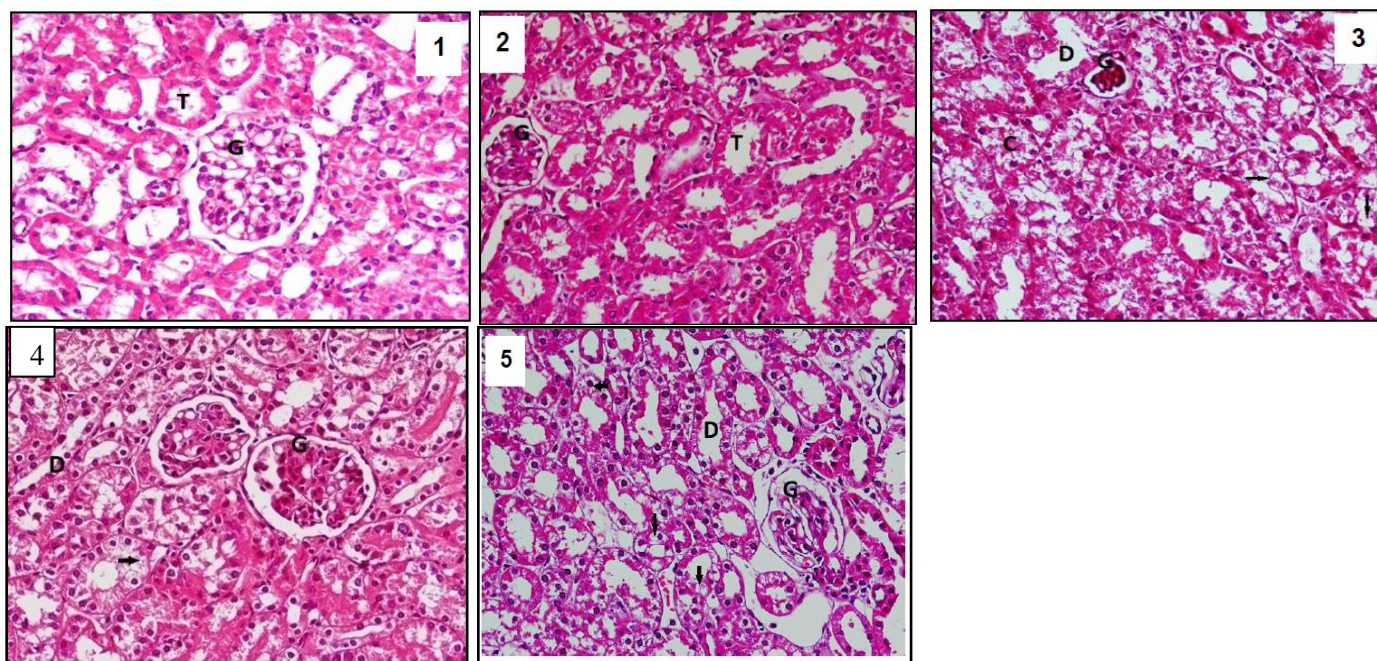


Figure 3. shows the effect of SPC and Diamicron®30 MR administration on the kidney tissue of STZ-induced diabetic rats in compare to that of normal control (H&E, x400). 1) normal rats show normal appearance of the glomeruli (G) and tubules (T); 2) normal-SPC treated animal showed normal appearance of the glomeruli (G), and the tubules (T); 3) diabetic untreated animal; showed moderately shrunken glomeruli (G), dilated tubules (D), wide tubular cells (arrows) and some intratubular casts (C); 4) diabetic-Diamicron®30 MR treated group; the glomeruli showed mild thickening of the basement membrane (G), occasional tubules are dilated (D) and some of the tubular cells showed vacuolization (arrow); and 5) diabetic-SPC treated group; it shows normal appearance of the glomeruli (G), few of the tubules showed mild dilatation (D), some of the tubular cells showed vacuolization of their cytoplasm.

Discussion

Our current study showed that soy protein intake increase insulin level. Similar to the present results, Lu et al.[18] reported that high-isoflavone soy protein administration to diabetic rats significantly increased serum insulin level and decreased blood glucose compared with the control or low-isoflavone soy protein groups. Previous studies have shown that the isoflavones genistein [19, 20] and to a lesser extent the isoflavones daidzein [20] increased

insulin secretion from islet preparations. Another study by Liu et al.[21] found that genistein increases glucose-stimulated insulin secretion in cell lines and mouse pancreatic islets at micromolar concentrations via a cAMP-dependent protein kinase mechanism.

Hyperglycemia that an essential reason for the release of free radicals [22], which develop the diabetic complications, reactive oxygen species (ROS) such as the superoxide radical anion and the peroxide non-radical

dianion can be produced both by means of enzymes and by non-enzymatic chemical reduction of molecular oxygen. ROS are highly reactive and attack in their vicinity various classes of biomolecules including proteins, DNA and lipids such as polyunsaturated fatty acids (PUFAs). This phenomenon is generally known as "oxidative stress" or "oxidant stress". The PUFA arachidonic acid is peroxidized to finally form malondialdehyde (MDA) [23,24]. This suggestion is asserted by our results that show significant increase in hepatic and renal MDA and nitric oxide due to STZ induced diabetes mellitus. The increased nitric oxide production is recognized as an important mediator of physiological and pathological processes [25].

Antioxidant enzymes as well as non-enzymatic antioxidants are first line of defense against ROS induced oxidative damage in a living organism [26]. SOD, CAT and GSH-Px are the three major enzymes that remove the toxic free radicals *in vivo* [27]. Glutathione functions as a free radical scavenger and is an essential cosubstrate for GSH-Px [28]. The decreased activity of antioxidant (SOD and CAT) enzymes along with decreased GSH level were found in renal and hepatic tissues of hyperglycemic rats in the current study. It was suggested that decreased antioxidant enzyme activity in diabetic individuals could be due to glycation of these enzymes, which occurred at persistently elevated blood glucose levels [29]. Endogenous antioxidants CAT, SOD and GSH act as reducing agents and detoxified highly reactive oxygen and nitrogen species [29]. The measured GSH, CAT and SOD are markedly depressed in STZ diabetic groups due to oxidative stress, this study results are agreed with the results of Sinzato *et al.* [30] & Basha and Saumya [31].

It was recently reported that elevated oxidative/nitrosative stress leads to fragmentation of nuclear DNA in liver, which contribute to hepatocellular apoptosis as well as necrosis [32]. Furthermore, DNA fragmentation is considered as a hallmark event in cell apoptosis. In our study, we evaluated the level of DNA damage through quantification of fragmented DNA. In our study, we reported a significant DNA fragmentation level ($P < 0.01$) quantified in hepatic tissues of STZ-induced diabetic rats potentially concurrent with significant increase in MDA and NO levels. DNA fragmentation increased approximately two fold in diabetic rat liver compared to the normal liver. It was evidenced previously that administration of graded doses of STZ to rats *in vivo* stimulated H_2O_2 generation and induced DNA fragmentation [33].

Oxidative stress is a major contributor to cardiovascular disease in diabetes mellitus, in fact it is well established that diabetes is associated with increased oxidative stress as evidenced by the excessive accumulation of lipid peroxides in the plasma of rats with diabetes mellitus so that hyperglycemia shows increase in total cholesterol, LDL-cholesterol and triglyceride levels and decreased

HDL level [34]. In diabetic state lipoprotein lipase, which hydrolyzes triglycerides, is not activated due to insulin deficiency, resulting in hypertriglyceridemia, and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities [34]. Triglycerides stimulate the secretion of very low-density lipoprotein cholesterol and such increase in very low-density lipoprotein cholesterol particles reduces the HDL-cholesterol level and increases the LDL-cholesterol particles [35]. In our study, the altered serum lipid profile was found in hyperglycemic group. This finding is in correlation with the findings of Al-Jamal and Alqadi [36]. Several reports indicated that hyperglycemia produces abnormally high levels of ROS, and these species could react with essential molecules such as lipids, proteins and DNA, leading to histological changes as well as functional alterations [37]. STZ toxicity is related to its ability to induce selective destruction of pancreatic beta cells resulting in insulin deficiency and hyperglycemia [38]. In the present study, reduction in body weight in diabetic rats was observed which might be the result of degradation of structural proteins due to unavailability of carbohydrates for utilization as an energy source [39,40]. Our results agree with previous observations that have also reported loss of body weight [23].

In STZ-induced diabetic rats, elevated serum levels of ASAT, ALAT and GGT, urea and creatinine were observed. This behavior suggested the occurrence of liver and kidney damages after the administration of STZ to the rats. The elevations in liver enzymes (ASAT, ALAT and GGT) are due to STZ mediated liver damage, which may cause leakage of these enzymes into the blood [41] due to loss of functional integrity of the hepatic cell membranes [42]. Hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment in the free radicals thereby depleting the antioxidant defense system and thus promoting de novo free radical generation that may lead to liver cell damage [43]. The increases in liver enzymes (ASAT and ALAT) in the current study are agreed with the results of Lin and Sun [44] & Mohammed *et al.* [45]. On the other hand, the persistent hyperglycemia, hemodynamic changes within the kidney tissue and free radical generation mediated renal dysfunction, which results in elevation of urea and creatinine levels in blood [46,47]. Our results show a marked increase in blood urea and creatinine due to STZ induced oxidative stress and free radicals that cause kidney damage and these results are going with the results of Wu *et al.* [48].

Soybean contains proteins, lipids, fiber and a variety of bioactive phytochemicals such as saponins, phytic acid, trypsin inhibitors, fiber and isoflavones. The isoflavones are the most active components of soy protein and though to be responsible for many of its beneficial effects on blood lipids of humans and animals [49].

Administration of soy protein to STZ-induced diabetic animal group showed a significant decrease ($P < 0.01$) in liver DNA fragmentation % as compared to diabetic group. The reduced level of DNA damage in soy protein treated diabetic animals may be attributed to the antioxidant activity of the soy proteins [50, 51]. This result was supported with our data of remarkable reduced levels of MDA and NO combined with elevation of SOD, CAT, and GSH in soy protein treated animals. This ability of soy protein, administered orally, to prevent oxidative DNA damage in the liver of diabetic rats should contribute to its use in the management of diabetes.

In contrast, Diamicon treatment for diabetic animals increased remarkably ($p < 0.01$) the level of fragmented DNA level compared to diabetic group. Desfaits *et al.* [52] & Chitturi *et al.* [53] mentioned that gliclazide [Diamicon], a second generation sulfonylurea used in the treatment of type 2 diabetes mellitus, has antioxidant properties, but it also induced acute hepatitis and liver toxicity.

Soy protein, compared with animal protein sources in humans and animals has a hypolipidemic potential. Afterwards, many studies suggest that the lipid lowering effect of soy protein is mainly attributed to its protein content [54]. It was found by Sirtori *et al.* [55] & Tachibana *et al.* [56] that rats fed soy protein had lower serum cholesterol levels and decreased liver triacylglycerol content caused by the STZ. The results of Tachibana *et al.* [56] & Bakhit *et al.* [57] study demonstrate that soy protein intake induced a marked decrease in the levels of serum cholesterol and triglycerides and they suggested that soy protein down-regulates genes involved in cholesterol synthesis.

The results of our study show that soy protein ameliorate liver and kidney functions in diabetic rats, this effect may be due to improvement in serum free fatty acids and serum fat levels [27] which may attributed to the soy protein constituents, such as amino acid profile, peptides, or isoflavones [27]. Soy isoflavones have antioxidant properties [58]. So, GSH, CAT and SOD levels were restored toward normal levels in soy treated diabetic rats, and therefore, decreased MDA level, restored liver integrity and improvement in liver enzymes (ASAT and ALAT) were observed in diabetic rats treated with soy protein.

After long time, hyperglycemia leads to chronic renal vasodilatation, glomerular hyperfiltration, cellular injury, glomerulosclerosis, mesangial proliferation, and also, proteinuria and uremia [59]. Previous studies have suggested that soy protein intake instead of animal protein will protect against development of kidney disease in diabetic individuals and be effective in reversing or slowing the progression of established kidney disease in diabetic individuals [60]. Our study showed that soy protein treatment significantly decreased the level of serum creatinine and urea and these results

cope with the results of Chen *et al.* [61] & James and Anderson [62].

The biochemical study was further confirmed by the histopathological examination of both liver and kidney tissues. Liver sections of both normal and normal-SPC treated animal groups displayed normal architecture and appearance of portal tract and hepatocytes. Similarly, their kidneys showed a normal appearance; the glomeruli illustrated normal cellularity and normal thickening of the basement membranes; the tubules are normal with no abnormal dilatation and no intraluminal casts were noticed; and the tubular cells showed no vacuolization. This finding reflects the safe effect of soy and goes in line with Sarhan *et al.* [63]. Unlikely, liver sections from diabetic rats group showed severe injury illustrated in mononuclear cell infiltrate extending through hepatic tissue. Kupffer cell appeared engulfing debris and hyperplasia of bile duct. Obviously fatty change which is also a common feature could be seen giving cloudy appearance. This finding is in accordance with Waer and Helmy [64]. Also, kidney section of STZ-induced diabetic rats showed shrunken glomeruli with thickened basement membranes; markedly dilated tubules with intratubular casts and many of the tubular cells showed cytoplasmic vacuolization. Interestingly, diabetic-SPC treated animals showed improvements of many pathological features compare to the diabetic ones, however normal appearance of the glomeruli, few tubular mild dilatation, and few tubular cellular cytoplasmic vacuolization. However diabetic-diamicon@30 MR treated animals still illustrate some pathological features in the form of thickening of the basement membrane of the glomeruli, occasional dilatation of the tubules with some of the tubular cells showed vacuolization of their cytoplasm. This result is in concomitants with Eoh and Abd Latiff [65].

The STZ diabetic rats exhibited persistent hyperglycemia which is the main diabetogenic factor and contributes to the increase in oxygen free radicals by autoxidation of glucose. Hyperglycemia also generates reactive oxygen species, which in turn, cause lipid peroxidation and membrane damage. Diabetes increases oxidative stress in many organs, especially in the liver [66]. It was suggested that hyperglycemia, through increasing in advanced glycation end products, facilitates free radicals production via disturbance in ROS production. Also, it was reported that loss of insulin following streptozotocin treatment resulted in caspase- and c-Jun N-terminal kinase (JNK)-dependent liver injury [67]. Thus, a suitable drug must have both antioxidant and blood glucose decreasing properties [68-70]. Soy protein concentrate improved the STZ/diabetic-induced hepatic and renal histological deteriorations; these effects are confirmed by the reports of Sarhan *et al.* [63] and Fair *et al.* [71] and the biochemical and DNA fragmentation results of this study. A mixture of compounds rather than a single compound

may be the key to full antioxidant potency; consequently, the antioxidative effects of SPC reported in the current study may be due to its several active compounds. In conclusion, the protective effects of SPC against STZ-induced diabetes mellitus may be attributed to its antioxidant and free radical scavenging activities due to its higher contents of isoflavones and antioxidant components.

Conclusion

Soy protein concentrate exhibited valuable hypoglycemic and highly protective effects against STZ-induced diabetes mellitus; these potentials may be achieved via the antioxidant and free radical scavenging mechanisms those due the higher contents of thiol-rich amino acids and other antioxidant ones.

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